**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| Reexam Control No. 90/008,268 | ) | |
| | ) | Group Art Unit: 3991 |
| Patent No. : 6,719,840 B1 | ) | |
| (Application No. 09/877,405, filed June 8, 2001) | ) | Examiner: Jerry D. Johnson |
| | ) | |
| Issued: April 13, 2004 | ) | |
| | ) | |
| Title: IN SITU CRYSAL GROWTH AND CRYSTALLIZATION | ) | |

**CERTIFICATE OF ELECTRONIC TRANSMISSION UNDER 37 C.F.R. 1.8**

I hereby certify that this correspondence, including listed enclosures, is being electronically transmitted to the United States Patent and Trademark Office in accordance with 37 C.F.R. 1.6(a)(4) on:

Dated: 12/19/07

Signed: /Jan Valla/

## RESPONSE TO EX PARTE REEXAMINATION COMMUNICATION

An Ex Parte Reexamination Communication was mailed in regard to the above referenced reexamination on October 19, 2007.

The Patent Owner's Response to such Ex Parte Reexamination Communication in accordance with 37 CFR § 1.52(a) and (b) is provided within two months of the date of such communication. The Commissioner is hereby authorized to charge any required fees to Deposit Account No. 50-0310 (Docket 067450-5000).

The issued claims begin on page 2. Patent Owner's Response begins on page 7 herein.

## Claims

Claim 1 (issued): A method for determining crystallization conditions for a protein, the method comprising: within a microfluidic device, delivering material to an enclosed microvolume via one or more lumens that each have a cross sectional diameter of less than 500 microns to form a plurality of different crystallization samples within the enclosed microvolume, the plurality of different crystallization samples comprising a protein to be crystallized and crystallization conditions which vary among the plurality of different crystallization samples; allowing crystals of the protein to form in the plurality of crystallization samples within the microfluidic device; and identifying which of the plurality of crystallization samples within the microfluidic device comprise a precipitate or a crystal of the protein.

Claim 2 (issued): A method according to claim 1 wherein the en closed microvolume is a lumen.

Claim 3 (issued): A method according to claim 1 wherein the enclosed microvolume is a lumen with a cross sectional diameter of less than 2.5 mm.

Claim 4 (issued): A method according to claim 1 wherein the enclosed microvolume is a lumen with a cross sectional diameter of less than 1 mm.

Claim 5 (issued): A method according to claim 1 wherein the enclosed microvolume is a lumen with a cross sectional diameter of less than 500 microns.

Claim 6 (issued): A method according to claim 1 wherein the enclosed microvolume is a microchamber.

Claim 7 (issued): A method according to claim 1 wherein the enclosed microvolume is at least partially enclosed within a substrate which comprises other enclosed microvolumes which also comprise crystallization samples.

Claim 8 (issued): A method according to claim 1 wherein the enclosed microvolume is at least partially enclosed within a card shaped substrate.

Claim 9 (previously presented): A method according to claim 1, the method further comprising performing a spectroscopic analysis on a precipitate or crystal formed within the microvolume.

Claim 10 (issued): A method according to claim 9, wherein the spectroscopic analysis is selected from the group consisting of Raman, UV/VIS, IR, and x-ray spectroscopy.

Claim 11 (issued): A method according to claim 9, wherein the spectroscopic analysis is x-ray spectroscopy.

Claim 12 (issued): A method according to claim 11, wherein x-ray spectroscopy is performed such that a portion of the microvolume that the x-ray beam traverses contains at least as many electrons as is contained in a material defining the portion of the microvolume that the x-ray beam traverses.

Claim 13 (issued): A method according to claim 11, wherein x-ray spectroscopy is performed such that a portion of the microvolume that the x-ray beam traverses contains at least three times as many electrons as is contained in a material defining the portion of the microvolume that the x-ray beam traverses.

Claim 14 (issued): A method according to claim 11, wherein x-ray spectroscopy is performed such that a portion of the microvolume that the x-ray beam traverses contains at least five times as many electrons as is contained in a material defining the portion of the microvolume that the x-ray beam traverses.

Claim 15 (issued): A method according to claim 11, wherein x-ray spectroscopy is performed such that a portion of the microvolume that the x-ray beam traverses contains at least ten times as many electrons as is contained in a material defining the portion of the microvolume that the x-ray beam traverses.

Claim 16 (issued): A method according to claim 1, wherein material defining the microvolume defines a groove that reduces a number of electrons that an x-ray beam used to perform x-ray spectroscopy of a crystal within the microvolume traverses in the process of performing x-ray spectroscopy on the sample within the microvolume.

Claim 17 (issued): A method according to claim 1, wherein the method further comprises forming the plurality of different crystallization samples within the enclosed microvolume.

Claim 18 (issued): A method according to claim 1, wherein one or more dividers are positioned within the enclosed microvolume to separate adjacent crystallization samples within the enclosed microvolume.

Claim 19 (issued): A method according to claim 18, wherein the one or more dividers are formed of an impermeable material.

Claim 20 (issued): A method according to claim 18, wherein the impermeable material is an impermeable liquid.

Claim 21 (issued): A method according to claim 18, wherein the impermeable material is an impermeable solid.

Claim 22 (issued): A method according to claim 18, wherein the one or more dividers are formed of a permeable material.

Claim 23 (issued): A method according to claim 18, wherein the one or more dividers are formed of a semipermeable material.

Claim 24 (issued): A method according to claim 23, wherein the semipermeable material is a gas.

Claim 25 (issued): A method according to claim 23, wherein the semipermeable material is a liquid.

Claim 26 (issued): A method according to claim 23, wherein the semipermeable material is a gel.

Claim 27 (issued): A method according to claim 18, wherein at least one of the one or more dividers form an interface selected from the group consisting of liquid/liquid, liquid/gas interface, liquid/solid and liquid/sol-gel interface.

Claim 28 (issued): A method according to claim 18, wherein the one or more dividers are selected from the group consisting of a membrane, gel, frit, and matrix.

Claim 29 (issued): A method according to claim 18, wherein the one or more dividers function to modulate diffusion characteristics between adjacent crystallization samples.

Claim 30 (issued): A method according to claim 18, wherein at least one of the one or more dividers is formed of a semipermeable material which allows diffusion between adjacent crystallization samples.

Claim 31 (issued): A method for determining crystallization conditions for a protein, the method comprising: within a microfluidic device, delivering material to a plurality of enclosed microvolumes via one or more lumens that each have a cross sectional diameter of less than 500 microns to form a plurality of different crystallization samples within the plurality of enclosed microvolumes, each microvolume comprising two or more crystallization samples, the different crystallization samples comprising a protein to be crystallized and crystallization conditions which vary among the plurality of different crystallization samples; allowing crystals of the protein to form in the plurality of crystallization samples; and identifying which of the plurality of crystallization samples comprise a precipitate or a crystal of the protein.

Claim 32 (issued): A method according to claim 11, wherein the x-ray spectroscopy is x-ray diffraction.

Claim 33 (issued): A method according to claim 11, wherein x-ray spectroscopy is performed such that a portion of the crystal or precipitate that the x-ray beam traverses contains at least as many electrons as is otherwise traversed by the x-ray beam when traversing a device comprising the microvolume.

Claim 34 (issued): A method according to claim 11, wherein x-ray spectroscopy is performed such that a portion of the crystal or precipitate that the x-ray beam traverses contains at least three times as many electrons as is otherwise traversed by the x-ray beam when traversing a device comprising the microvolume.

Claim 35 (issued): A method according to claim 11, wherein x-ray spectroscopy is performed such that a portion of the crystal or precipitate that the x-ray beam traverses contains at least five times as many electrons as is otherwise traversed by the x-ray beam when traversing a device comprising the microvolume.

Claim 36 (issued): A method according to claim 11, wherein x-ray spectroscopy is performed such that a portion of the crystal or precipitate that the x-ray beam traverses contains at least ten times as many electrons as is otherwise traversed by the x-ray beam when traversing a device comprising the microvolume.

Claim 37 (issued): A method according to claim 31, wherein each microvolume comprising a plurality of crystallization samples.

Claim 38 (issued): A method according to claim 11, wherein x-ray spectroscopy is performed such that a portion of the microvolume that the x-ray beam traverses contains at least half as many electrons as is contained in a material defining the portion of the microvolume that the x-ray beam traverses.

## Patent Owner's Response

The Patent Owner will address the substantial questions of patentability raised in the Ex Parte Reexamination Communication in the order in which such issues were presented in the communication.

### 1. Patentability of Claims 1-11, 17, 31, 32 and 37 Over U.S. Patent No. 6,409,832 to Weigl et al. (Weigl) and U.S. Patent 6,899,137 to Unger et al. (Unger)

In the Communication, the Examiner rejected claims 1-11, 17, 31, 32 and 37 as being unpatentable over U.S. Patent No. 6,409,832 to Weigl et al. (Weigl) under 35 U.S.C. §102(b). The Examiner also rejected claims 1-11, 17, 31, 32 and 37 as being unpatentable over U.S. Patent No. 6,899,137 to Unger et al. (Unger) under 35 U.S.C. §102(e).

The Patent Owner respectfully traverses both of these rejections on the grounds that neither reference teaches formation of a plurality of different crystallization samples in a single enclosed microvolume.

#### (a) Claim Construction of Independent Claims 1 and 31

Independent claims 1-31 require formation of a plurality of different crystallization samples in a single enclosed microvolume. Neither Weigl nor Unger teach this.

The Examiner's attention is drawn to independent claim 1 which specifies

> delivering material to **an** enclosed microvolume ... to form a plurality of different crystallization samples within **the** enclosed microvolume.

The Examiner's attention is also drawn to independent claim 31 which specifies "delivering material to a plurality of enclosed microvolumes... to form a plurality of different crystallization samples" where "**each microvolume** compris[es] two or more crystallization samples."

Despite the above noted language of independent claims 1 and 31, the Examiner maintained the rejections based on Weigl and Unger stating at page 5 of the Official Action that

> the term 'enclosed microvolume' ***(Note use of singular)*** thus includes microfluidic structures ***(Note use of plural)*** containing separate and discrete crystallization volumes.

The Patent Owner disagrees with this definition. "An enclosed microvolume" means a **single** microfluidic structure ***(Note use of singular)***. The term, as used in US Patent No. 6,719,840, does not mean an entire microfluidic device encompassing multiple lumens and microchambers.

The Patent Owner reminds the Examiner to interpret "an enclosed microvolume" based on the claims and Specification of US Patent No. 6,719,840. After all, the Patent Owner is entitled to be his own lexicographer. The Examiner's definition for enclosed microvolume cannot contradict the meaning the Patent Owner establishes through both the claims and the Specification.

The Specification teaches that lumens and microchambers each constitute **separate** enclosed microvolumes. Specifically, Col. 4, line 62–Col. 5, line 6 teaches

> According to the present invention, crystallization samples are formed, transported, and crystallization attempts conducted in enclosed microvolumes ***(Note use of plural)***. These enclosed microvolumes ***(Note use of plural)*** comprise one or more lumens and optionally microchambers in fluid communication with the lumens. The lumens are enclosed within a substrate. When employed, microchambers are enclosed microvolumes defined within the substrate in fluid communication with the lumens. The lumens and microchambers provide an encased environment within which crystallization samples may be formed, and crystallization attempts performed and analyzed.

By contrast to the above passage from the Specification which specify "enclosed microvolumes" ***(Note use of plural)***, the claims purposefully use the term "an enclosed microvolume" ***(Note use of singular)*** in order to specify formation of "a plurality of different crystallization samples within ***[a single]*** enclosed microvolume."

In view of the claim language and the teaching in the Specification, the Examiner cannot interpret "**an** enclosed microvolume" ***(singular)*** as the term is used in US Patent No. 6,719,840 to mean an entire microfluidic device encompassing multiple lumens and microchambers when attempting to support a prior art rejection. The Specification and claims of US Patent No. 6,719,840 are controlling as to the meaning of an enclosed microvolume and they make it clear that an enclosed microfluidic structure ***(singular)*** refers to a particular lumen or particular microchamber within a microfluidic device.

**(b) Traversal of Examiner's § 102(b) Rejection Based on Weigl**

In support of Weigl rejection, the Examiner states that Weigl teaches a

> method comprising delivering material to an enclosed microvolume ***(Note use of singular)*** via one or more lumens to form a plurality of different crystallization samples with varying crystallization conditions.

Official Action, page 3.

Based on a proper definition for "an enclosed microvolume," Weigl does **not** teach that a "plurality of different crystallization samples with varying crystallization conditions" can be formed in the same enclosed microvolume.

The Examiner states that

> Weigl further teaches the application of three different approaches to crystallization capable of being performed simultaneously in a single enclosed microvolume.

The Patent Owner agrees that "Weigl… teaches the application of three different approaches to crystallization."

Weigl teaches three different approaches to crystallization, namely

(1) Diffusion based crystallization

See Col. 11, line 54 – Col. 12, line 8; Figure 1; and
Col. 13, line 66 – Col. 14, line 7; Figure 7;

(2) Premixing based crystallization

See Col. 12, lines 9-29; Figure 2; and

Col. 14, lines 18-27; Figure 8;

(3) Crystallization by increasing the concentration of protein

See Col. 12, lines 30-55; Figure 3;

Col. 14, lines 28-35; Figure 9.

In each instance, Weigl is very specific where crystallization occurs, namely:

(1) Diffusion based crystallization occurs at diffusion interface zones 16, 18 within channel 15 (Col. 11, lines 63-66) and crystallization chamber 39 (Col. 14, line 5);

(2) Premixing based crystallization occurs at crystallization channel 15 (Col. 12, lines 17-18) and crystallization chamber 39 (Col. 14, lines 23-24); and

(3) Crystallization by increasing the concentration of protein occurs at crystallization channel 15 (Col. 12, lines 30-31) and crystallization chamber 39 (Col. 12, line 30).

In order to meet the requirements of claims 1 and 31, Weigl **must** teach that "plurality of different crystallization samples with varying crystallization conditions" can be performed in either:

(1) diffusion interface zones 16, 18 of Figure 1;
(2) crystallization chamber 39 of Figure 7;
(3) crystallization channel 15 of Figure 2;
(4) crystallization chamber 39 of Figure 8;
(5) crystallization channel 15 of Figure 3; or
(6) crystallization chamber 39 of Figure 9.

Weigl does not provide this required teaching.

Although Weigl teaches that three different approaches to crystallization may simultaneously be performed in **a single microfluidic device**, Weigl does **not** teach that the

three different approaches to crystallization may be simultaneously performed in a **single enclosed microvolume** as required by independent claims 1 and 31. Rather, Weigl teaches that the three different approaches may be used in parallel on a single substrate:

The Examiner cites to Column 13, lines 55-65 of Weigl to support Examiner's statement that

> Weigl further teaches the application of three different approaches to crystallization capable of being performed simultaneously in a single enclosed microvolume.

The cited Column 13, lines 55-65 of the patent recites:

> Three approaches can be used in the microfluidic circuit cartridges to initiate protein crystallization and accompanying figures show the conceptual design for a single PCG experiment on the prototype board. *It should be noted that all three approaches could be mixed and matched onto a single board.* The PCG techniques are: self-diffusion of precipitants and protein across a laminar boundary (see FIG. 7); turbulent mixing of all components--batch mode (see FIG. 8); and vapor transport into a desiccant or precipitant (see FIG. 9). Emphasis added.

This above section of Weigl indicates that the layout of the overall microfluidic circuit cartridges can include multiple different types of individual microfluidic circuits. Figures 7-9 teach three different types of microfluidic circuits for performing crystallizations. However, it is clear from Weigl that the particular enclosed microvolume of each microfluidic circuit where crystallization occurs (i.e., crystallization chamber 39 of Figures 7-9) only contains a single different crystallization sample. Hence, Col. 13, lines 55-65 does not support the Examiner's rejection.

Therefore, while Weigl teaches three different techniques, Weigl does not teach using more than one technique in a single enclosed microvolume. In fact, Figure 11 of Weigl shows a board with multiple, unconnected volumes and states that the microfluidic device of Figure 11 is a "microfluidic cartridge for performing high density screening crystallization". See column 9, lines 11 and 12. Similarly, Figure 5 depicts a plurality of crystallization chambers, however, Weigl makes clear each are separate volumes:

> Referring now to FIG. 5, a microfluidic cartridge, generally indicated at 50, contains a plurality of fluid reservoirs 32, 34. Reservoirs 32 are filled with a protein sample, while reservoirs 34 are filled with a precipitant solution. Fluids in reservoirs 32, 34 are expelled by applying pressure to a fluid located within channel 30, which may be air or an inert oil. Reservoirs 32, 34 combine to form a T-sensor structure with **crystallization chamber 39**. Laminar flow ensures that the two fluids do not mix within chamber 39 other than by mutual self-diffusion. The contents of crystallization chamber 39 void into harvesting chamber 40. Each fluid reservoir 32, 34 is filled through a fluid inlet 52 and has microfluidic channel/check valves 36, 38 a vent hole 54 to permit air escape during the filling operation. Surface tension effects because of the small diameter of the connecting to the fluid reservoirs 32, 34 prevent fluids flowing out of said reservoirs. Once loaded, fluid reservoirs 32, 34 are carefully sealed with adhesive strip 44, as can be seen in FIG. 6. (Emphasis added).

As can be seen from the above passage and with reference to Figure 5, each crystallization chamber (39) is separate from the others.

> The Examiner also cites to Column 16, lines 5-15 of Weigl which recites The microfluidic integrated circuit cartridges, when sealed with the covering adhesive film, comprise one level of fluid containment. The fluid driver interface connection on the circuit cartridges is airtight, while the air bellows design does not compromise the containment level. Fifty (50) microfluidic integrated circuit cards containing up to 20 individual PCG experiments each or 1000 PCG experiments in all could fit with external controllers into a sealed container within the volume of a mid-deck locker that provides the second level of containment and, if required, temperature control.

This above quoted section of Weigl teaches that

(a) a given microfluidic integrated circuit cartridge contains a **plurality** of microfluidic circuits (same teaching as Col. 13, lines 55-65), and

(b) multiple microfluidic integrated circuit cartridges can be connected together.

This further citation to Weigl does not overcome the fundamental deficiency of Weigl as a 35 USC 102(b) reference.

As the Examiner is aware, it is well established that "[a] claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." Verdegaal Bros. v. Union Oil Co. of California, 2 USPQ 2d 1051, 1053 (Fed. Cir. 1987), cert. denied, 481 U.S. 1052 (1987). See also, Scripps Clinic and Research Foundation v. Genentech, Inc., 18 USPQ 2d 1001 (Fed. Cir. 1991).

In the present case, Weigl does not teach a microfluidic circuit for conducting crystallizations where a particular enclosed microvolume in which crystallization occurs (i.e., crystallization chamber 39 of Figures 7-9) contains a plurality of different crystallization samples. Instead, because the particular microvolume in which crystallization occurs according to Figures 1-3, 5 and 7-9 only contains a single crystallization sample, the Examiner's rejection is unsupported and should be withdrawn.

**(c) Traversal of Examiner's §102(e) Rejection Based on Unger**

Like Weigl, Unger is very specific where crystallization occurs, namely:

> Each of flow channels 7204a, 7204b, 7204c, and 7204d feature dead-end chambers 7206 that serve as the site for recrystallization.

See Col. 53, line 66 - Col. 54, line 1.

There is no teaching in Unger regarding how to make a given dead-end chamber 7206 contain a plurality of different crystallization samples. In this regard, the Examiner's attention is drawn to Col. 55, lines 4-10 and 21-30 of Unger which teaches:

> Operation of protein crystallization system 7500 is as follows. Initially, an aqueous solution containing **the target protein is flushed through each of flow channels 7504a, 7504b, 7504c, and 7504d, filling dead-end chambers 7506**. Next, a high pressure is applied to control channel 7502 to actuate stop valves 7503, thereby preventing fluid from entering or exiting chambers 7506. [Unger, Col. 55, lines 4-10]
>
> Thus, when pressure is released from first control line 7502 and stop valves 7503 open, a different volume of countersolvent from the various segments 7514 **may diffuse into chambers 7506**. In this manner, precise dimensions defined by photolithography can be employed to determine

> the volume of countersolvent trapped in the flow channel segments and then introduced to the protein solution. This volume of countersolvent in turn establishes the environment for crystallization of the protein. [Unger, Col. 55, lines 21-30]

In addition, Patentees note that one of passages cited by the Examiner, Column 32, lines 20-68, are directed entirely to the synthesis of biopolymers such as DNA and proteins, and are not relevant to protein crystallization reactions at all.

As can be seen from the above quoted passages, Unger does not, however, teach how to conduct a plurality of different crystallization experiments in a given dead-end chamber 7506. Hence, like Weigl, Unger does not anticipate independent claims 1 and 31.

## 2. Patentability of Claims 9-11 and 32 Over Unger in view of Weigl or Chayen et al. "Trends and Challenges in Experimental Macromolecular Crystallography" (Chayen)

As discussed above, neither Weigl nor Unger teaches or suggests forming a plurality of different crystallization samples within a single enclosed microvolume. The other references do not make up for this gap in teaching. Accordingly, the Examiner is respectfully requested to withdraw this ground of rejection.

As discussed previously, Patent Owner amended claim 9 to specify "performing a spectroscopic analysis on a precipitate or crystal [formed] within the microvolume" in order to clarify that the claim further specifies performing the spectroscopic analysis while the precipitate or crystal **is still within the microvolume**.

Although Weigl teaches spectroscopic analysis of protein crystals, Weigl is not explicit with regard to where that analysis occurs. It would be understood by one of ordinary skill in the art that the harvesting chamber 40 is used to allow the crystals to be removed from the microfluidic device and analyzed outside of the device. Weigl therefore cannot be said to teach or suggest performing spectroscopic analysis within a microfluidic device.

As conceded by the Requester in the Request, Unger does not teach spectroscopic analysis of protein precipitate or crystals and certainly does not teach performing spectroscopic analysis within a microfluidic device.

Although Chayen may teach performing spectroscopic analysis of protein crystals, these references do not teach use of microfluidic device and thus cannot possibly teach performing spectroscopic analysis of protein crystals **within a microfluidic device**. The Patent Owner respectfully requests that the Examiner give patentable weight to this distinction, particularly in view of the teaching of the harvesting chamber 40 in Weigl. For this further reason, withdrawal of this ground of rejection is respectfully requested.

## 3. Patentability of Claims 12-16, 33-36 and 38 Over Unger, Weigl, and Chayen, in further view of US Patent 6,258,331 to Sanjoh (Sanjoh)

As discussed above, neither Weigl nor Unger teaches or suggests forming a plurality of different crystallization samples within a single enclosed microvolume. The other references do not make up for this gap in teaching. Accordingly, the Examiner is respectfully requested to withdraw this ground of rejection.

Claims 12-16, 33-36 and 38 also depend from dependent Claim 9 which the Patent Owner further distinguishes over the cited art in Section 2 above.

For these reasons, withdrawal of this ground of rejection is respectfully requested.

## CONCLUSION

The Patent Owner submits that all claims of the patent are patentable over the art relied upon by the Requester and respectfully request that the Examiner find that no substantial new question of patentability exists as to any of the claims. If the Examiner has any questions, the Examiner is requested to contact the undersigned attorney.

Respectfully submitted,
TAKEDA SAN DIEGO, INC.

Dated: December 19, 2007

By:/Robin Silva/
Robin M. Silva, Reg. No. 38,304 for
David J. Weitz, Reg. No. 38,362

Customer No. **32793**
Takeda San Diego, Inc.
10410 Science Center Drive
San Diego, CA 92121
Tel: (858) 622-8528
Fax: (858) 550-0992

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CERTIFICATE OF MAILING

I hereby certify that a true and correct copy of this Amendment and Patent Owner's Statement is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the following:

R. Danny Huntington, Esq.
BINGHAM McCUTCHEN LLP
Three Embarcadero Center, 18th Floor
San Francisco, CA 94111-4067

December 19, 2007
Date of Deposit

Jere Valles